signalling may be implicated in cartilage degeneration. Our previous studies demonstrated that abnormal mechanical load induces the nuclear translocation of β-catenin, indicating activation of the canonical WNT pathway. The purpose of this current study is to identify which WNT signalling components are regulated by mechanical load and how their regulation impacts on cartilage matrix homeostasis.

Methods: Methods: Full-depth articular cartilage explants were collected from the metacarpophalangeal joint of bovine calves and stabilised, in culture, for 72 hours prior to loading. Using the BOSE ElectroForce 3200® cartilage explants were either left unloaded or subjected to a physiological load of 2.5MPa (maintains cartilage turnover) or a degradatory load of 7MPa (1Hz, 15 minutes) (n=6 explants per parameter). WNT signalling PCR arrays (Qiagen, UK) were performed on RNA extracted from explants 2, 4, 8 and 24 hours post-load; data were normalised to the housekeeping genes SDHA and YWHAZ and further normalised to the unloaded explants. Mechano-regulation of identified genes of interest was validated using quantitative PCR. Expression of NFATc1, a mechanically regulated gene of interest, was subsequently inhibited using 25μM NFATc inhibitor (VIVIT peptide; Tocris) and explants subjected to load prior to downstream analyses.

Results: Results: As expected, the early response genes: FOSL1, JUN and MYC were significantly elevated in a load-dependent response peaking at 4hrs post-load (2.5MPa: 12.9-fold, 3.5-fold and 3.7-fold respectively and 7MPa: 68-fold, 10.5-fold and 14.1-fold respectively) before returning to basal levels by 24hrs. A cohort of genes were significantly down-regulated in a load-dependent manner at 4hrs including the WNT inhibitors DKK1 (2.5MPa: 4.1-fold; 7MPa: 10.2-fold) and PRICKLE1 (2.5MPa: 4.9-fold; 7MPa: 6.3-fold). Genes which were only modulated in response to the 7MPa degradative load included DKK2 (2.8-fold decrease) and WISP1 (2.3-fold increase). At 4hrs post-load, the transcription factors NFATC1 and TCF7 were also increased in a load-dependent manner. Quantitative PCR validation of the array findings indicated that NFATc1 transcription was elevated 2hrs post-load (2.5MPa: 10.6-fold; 7MPa: 26.6-fold) and further elevated at 4hrs post-load (2.5MPa: 26-fold; 7MPa: 32.7-fold); even at 24hrs post-load, NFATc1 transcription was still 2-fold higher than unloaded control explants. Treatment of articular cartilage with an NFATc inhibitor ameliorates load-induced expression of a number of Wnt-signalling genes indicating NFATc regulates both the canonical and non-canonical pathways.

Conclusions: Conclusion: Interestingly, several key WNT inhibitors including DKK1 and DKK2 are decreased in response to load, with further transcriptional inhibition as the load applied increases. DKK1 expression has been reported to be decreased in osteoarthritis; the mechano-regulation of this molecule might have implications for cartilage degeneration. Furthermore, the novel observation of NFATc1 mechano-regulation in articular cartilage may provide a further mechanism for maintaining load-induced tissue homeostasis. It has been reported that NFATc deficiency causes osteoarthritis, but conflicting evidence has suggested that NFATc signalling induces catabolism in chondrocytes. Collectively, this makes a compelling case for a role of NFATc proteins in maintaining cartilage joint health; future studies will delineate how mechanical load regulates the NFATc proteins and downstream components of this pathway to further our understanding of load-induced cartilage degeneration.

60
KNEE PAIN IS NOT RELATED TO ALTERATIONS IN THE MORPHOLOGY OR MRI SIGNAL OF THE INFRA-PATELLAR FAT PAD (IPFP) – A WITHIN-PERSON AND BETWEEN-PERSON ANALYSIS USING DATA FROM THE OSTEOARTHRITIS INITIATIVE (OAI)

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Purpose: Obesity is a known risk factor of OA, and this has been suspected to be at least partly conveyed by endocrinological mechanisms, i.e. fat cells secreting (pro-) inflammatory cytokines. The infrapatellar fat pad (IPFP) represents an accumulation of intra-articular adipose tissue and has been shown to represent a potential mediator of intra-articular inflammation. Inflammation was shown to be strongly associated with knee pain in OA, but the role of the IPFP in symptomatic knee OA has remained ill defined. The purpose of this study therefore was to investigate the quantitative imaging parameters of IPFP morphology and MRI signal intensity a) between painful and contralateral pain-free limbs of subjects with unilateral knee pain, and b) between knees of subjects with chronic pain vs. matched pain-free controls.

Methods: For the between-knee, within-person comparison, 46 subjects with unilateral pain were drawn from 4,796 OAI participants: both knees had to display the same radiographic stage (KLG2 or 3) but one knee had to display frequent pain (most days of the month within the past 12 months) and the contra-lateral one no pain. For the between-person comparison, 43 subjects with chronic pain (NRS≥4 and frequent pain at baseline, 2, and 4-year follow-up) were drawn from the OAI and were compared with control subjects without pain at all three time points (NRS≤1, no or infrequent pain) who were matched 1:1 by sex, age, height, weight, limb dominance, and radiographic disease stage (KLG). In the above subjects, the IPFP was segmented using sagittal intermediately weighted fat-suppressed turbo spin-echo images (slice thickness 3.0 mm, in-plane resolution 0.36 mm x 0.36 mm, TR=3200 ms, TE=30 ms), with all slices clearly depicting the IPFP being analyzed. The IPFP volume, its anterior surface (towards the lig. patellae), its mean thickness (depth) and the mean and standard deviation of the MRI signal intensity were then computed using custom software. Between-knee differences (baseline) and between-subject differences (at the 2-year follow-up time point) were tested for statistical significance using paired t-tests.

Results: Participants with unilateral knee pain were 67% female, 62.8±9.7 years old (mean±SD), and had a BMI of 30.0±4.7 kg/m^2. The IPFP volume in the painful knees was only very slightly greater (0.18 cm^3; 0.7%) than in painless knees but the 95% confidence interval was wide (Table 1, figure 1) and the difference was far from reaching statistical significance (p=0.64). Further, no significant differences were noted for the size of the anterior IPFP surface area, the IPFP depth, or the IPFP MRI signal intensity (mean and SD, table 1). Cases with chronic pain were 53% female, 60.7±9.0 years old, and had a BMI of 28.1±3.5 kg/m^2. Controls with bilaterally pain-free knees were matched 1:1 and hence displayed identical demographics. The IPFP volume of the chronically painful cases was only slightly lower than in matched painless controls (-0.54 cm^3; -2.1%, figure 1) and the difference was far

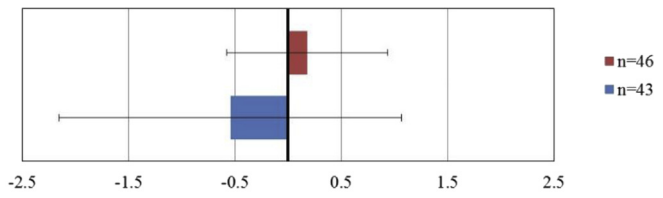
Table 1
IPFP morphometry and IPFP MRI signal in painful vs. painless knees within-person

Within-person design (n=46)	Case (Mean± SD)	Control (Mean± SD)	Difference (Mean± SD) (95% Confid. Int.)	Paired T-test
IPFP Volume [cm^3]	25.4±6.0	25.3±6.2	0.18±2.62 (−0.6,0.9)	p=0.641
Anterior surface [cm^2]	19.6±3.7	19.2±3.4	0.33±2.75 (−0.5,1.1)	p=0.426
Depth [mm]	13.0±1.9	13.0±1.7	-0.05±1.04 (−0.3,0.3)	p=0.768
Mean signal Intensity	154±27.2	149±35.4	5.40±32.9 (−4.1,14.9)	p=0.272
Signal Intensity SD	73.5±15.1	78.9±22.7	-5.39±19.5 (−11.0,0.3)	p=0.068

Table 2
IPFP morphometry and IPFP MRI signal in painful vs. painless knees between-person

Between-person design (n=43)	Case (Mean± SD)	Control (Mean± SD)	Difference (Mean±SD) (95% Confid. Int.)	Paired T-test
IPFP Volume [cm^3]	25.7±5.5	26.3±6.7	−0.54±5.39 (−2.2,1.1)	p=0.513
Anterior surface [cm^2]	19.2±3.2	18.7±4.2	0.46±3.81 (−0.7,1.6)	p=0.434
Depth [mm]	13.4±2.0	14.1±2.1	−0.63±2.50 (−1.4,0.1)	p=0.108
Mean signal Intensity	213±62.4	209±48.6	3.79±70.9 (−17.4,25.0)	p=0.728
Signal Intensity	110±30.5	102±22.5	7.60±36.5 (−3.3,18.5)	p=0.179

from being statistically significant (p=0.52; table 2). Further, no significant differences were observed in IPFP surface area, depth and MRI signal (Table 2).



Legend: Infra-patellar fat pad (IPFP) volume (cm³) differences and 95% CI of cases versus controls in a within person (n=46) and a between person (n=43) design

Figure 1. Infra-patellar fat volume [cm^3].

Conclusions: This is the first study to comprehensively address the relationship of human IPFP morphometry and MRI signal with knee pain. Although the sample size was limited, we obtained consistent results using a between-knee, within persons design, and a between person design with strict inclusion criteria of chronic pain and precise matching with painless participants. Although the IPFP has been shown to be a source and mediator of intra-articular inflammation, which is thought to be associated with pain, our results do not suggest a relationship between IPFP morphometry and MRI signal in human knee OA.

61
FUNCTIONAL MAGNETIC RESONANCE IMAGING EVALUATION OF PAIN CENTRAL SENSITIZATION PHENOMENA IN SUBJECTS WITH KNEE OSTEOARTHRITIS

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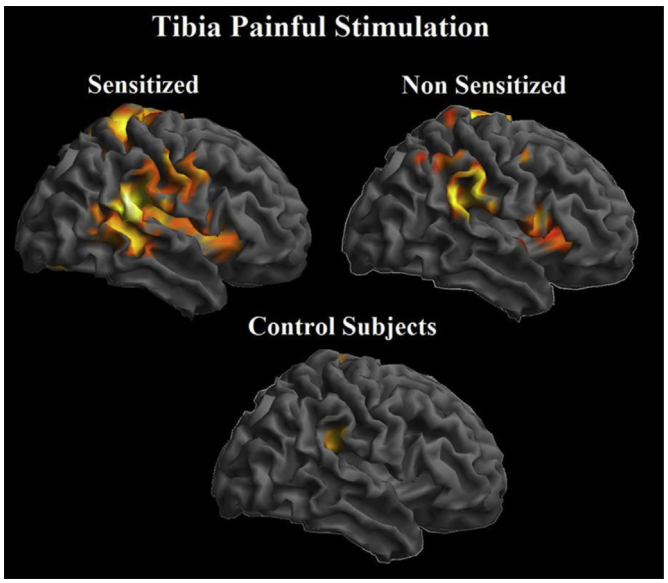
Purpose: The aim of this study was to characterize brain changes related to pain sensitization by functional magnetic resonance imaging (fMRI) in patients suffering from chronic osteoarthritis (OA) of the knee.
Methods: A cross-sectional, single blind study comparing fMRI activation in OA patients (clinical and radiological ACR criteria) vs. healthy controls was designed. Participants were consecutive recruited in follow-up clinical visits during a period of 18 months in the reference OA Unit at the Hospital del Mar in Barcelona. Presence of central sensitization was assessed in OA group. Central sensitization was clinically defined based on the evidence of regional spread of pain (spreading sensitization assessed by an extended version of the Arendt-Nielsen peripatellar map) and increased pain response to repeated stimulation (temporal summation). The fMRI paradigm included 3 painful test: direct painful stimulation of the knee (articular interline) using a pressure of 2.5kg/cm2, painful stimulation on the anterior tibial surface of the leg (sensitized site) by exerting a pressure of 4kg/cm2, and painful heat stimulation on the volar forearm using 45° Celsius peaks.
Results: A total of 60 patients (66.7 +/- 7.8 years) were included in the study along with a reference group of 30 healthy subjects (62.8 +/- 7.7 years). Thirty-three (55%) patients showed clinical evidence of sensitization, and 19 (32%) of them fulfilled strict experimental criteria of central sensitization to pain. fMRI response to articular (interline) painful stimulation was robust in both patients groups. Sensitized and non sensitized patients, however, did not differ as to brain activation during the interline test. By contrast, brain response to tibial pressure in sensitized patients was greater than in non-sensitized patients in several regions including the cortical representation of the leg (peak coordinates at x,y,z=-4,-42,60; t=3.31 and x,y,z=16,-38,64; t=3.74), the supramarginal gyrus (-48,-66,18; t= 3.24 and 52,-48,18 t= 3.42) and the right lower sensorimotor cortex (62,-8,40; t= 4.25).



Correlation maps between brain activation and pain sensitization measurements (the sum of the minimal pressure evoking pain for each tender point around the knee and the number of tender points) additionally showed the involvement of anterior brain regions, as the anterior cingulate gyrus (12,8,48; t=4.38) and mainly the ventral putamen bilaterally (-18,4,-8; t=3.7 and 20,18,-8; t=3.99). The painful heat stimulation on the volar forearm evoked similar subjective pain (no significant differences) and brain activation in both sensitized and non sensitized patients.
Conclusions: The presence of pain central sensitization in chronic knee OA patients was very common. As expected, the pressure at medial interline has shown not to be an appropriate test to discriminate sensitized patients, since it is a maneuver with direct impact on the damaged structures in the disease. Pressure stimulation on the (non-articular) anterior tibial surface of the leg, however, produced relevant clinical pain in sensitized patients and increased brain response. Pain brain sensitization was related to a widespread activation of sensory cortices suggesting that sensitization is expressed mostly as an enhanced sensory phenomenon. In addition, the changes in fronto-subcortical structures seen at correlation maps may speculatively suggest that pain central sensitization also involves alterations of elements implicated in associative pain-related learning (e.g., associations of pain with everyday contexts). Finally, negative results in the painful heat stimulation test suggest that sensitization is not a general phenomenon that may not implicate superior limbs or the processing of heat-elicited pain.

62
MOVEMENT CHARACTERISTICS ASSOCIATED WITH THE DEVELOPMENT OF CHRONIC KNEE PAIN

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Purpose: Substantial evidence suggests that previous knee injury is a risk factor for the development of knee osteoarthritis (KOA). Patellofemoral pain (PFP) is a chronic knee condition commonly caused by overuse injury, which is theorized to be associated with the development of KOA. While a direct link between PFP and KOA has yet to be established, there has been increased interest in this association in recent years. Gaining an understanding of the risk factors associated with the development of this chronic overuse injury may prove to be useful in the primary prevention of PFP, which may in turn reduce the occurrence of KOA. The aim of this study was to prospectively identify the baseline pre-injury movement patterns (e.g., kinematics) associated with the development of PFP.
Methods: We conducted a prospective cohort study that consisted of 4418 subjects (1662 females, 2756 males: 18.8±0.9yrs, 173.4±9.2cm,